THE JOURNAL OF INFECTIOUS DISEASES • VOL. 154, NO. 3 • SEPTEMBER 1986
 0022-1899/86/5403-0009$01.00

# Prevention of Experimental Coronavirus Colds with Intranasal α-2b Interferon

**Ronald B. Turner, Alma Felton, Kenneth Kosak, Douglas K. Kelsey, and Carlton K. Meschievitz**

*From the Departments of Pediatrics and Pathology, University of Utah School of Medicine, Salt Lake City, Utah; and the Schering Corporation, Kenilworth, New Jersey*

Fifty-five volunteers treated with either intranasal recombinant interferon (rIFN; $2 \times 10^6$ IU/day) or placebo for 15 days were exposed to coronavirus by direct intranasal inoculation on the eighth day of treatment. Symptom scores were recorded, and cultures of virus were taken daily for all volunteers for seven days after inoculation. Nineteen (73%) of the 26 placebo recipients met symptom-score criteria for a cold, compared with 12 (41%) of the IFN recipients ($P = .02$). The mean nasal symptom scores in the placebo and IFN groups were 9.2 and 5.4, respectively ($P = .03$), and the mean total symptom scores in the two groups were 23.2 and 9.4, respectively ($P = .003$). The mean number of days with a total symptom score >4 was 1.6 in the placebo recipients and 0.5 in the rIFN recipients ($P = .02$). Prophylactic intranasal rIFN effectively shortened the duration and reduced the severity of coronavirus cold symptoms.

Common colds occcur an average of two to four times per year per adult and six to 10 times per year per child [1, 2]. Recent studies have examined the efficacy of interferon prophylaxis for prevention of colds in the family setting [3, 4]. These studies indicate that rhinovirus colds can be effectively prevented. The effect on total respiratory illness, however, is less impressive and suggests that a major impact on the incidence of the common cold will require preventing infections caused by other viral pathogens.

Coronaviruses are believed to be responsible for at least 10% of upper-respiratory-tract illnesses [5], although information about coronavirus epidemiology is limited by the lack of reliable methods of isolating virus from clinical specimens. A recent report [6] suggests that several immunologically distinct strains of coronavirus exist that are capable of causing human respiratory tract infections. Infection confers protection to the homologous strain of virus; however, the duration of this immunity is not known. The existence of several viral strains and the uncertainty about the possibility of reinfection suggest that prevention of coronavirus infection by active immunization will be difficult. A previous study [7] and the observation that coronavirus 229E is sensitive to recombinant human α-2b interferon (rIFN) in vitro (authors' unpublished observations) suggest that intranasal interferon may be useful for treating or preventing coronavirus colds. The purpose of this randomized, double-blind study was to determine the efficacy of rIFN in preventing coronavirus colds in human volunteers.

Received for publication 13 November 1985, and in revised form 7 April 1986.

Informed written consent was obtained from all volunteers. The study was approved by the Institutional Review Board of the University of Utah and was conducted according to the guidelines of the U. S. Department of Health and Human Services.

This work was supported by the Schering Corporation, Kenilworth, New Jersey.

Please address requests for reprints to Dr. Ronald B. Turner, Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah 84132.

## Subjects and Methods

*Volunteers.* Healthy young adult volunteers were recruited from the University of Utah. Individuals who reported upper-respiratory-tract illness in the preceding week or who were taking oral or intranasal medications that would interfere with infection or assessment of symptoms were excluded. Each volunteer was documented to have a normal, complete blood cell count, blood chemistry, and urinalysis before participating in the study. No attempt was made to select antibody-free volunteers for the study. All but two volunteers had prechallenge titers of antibody >1:4.

*Interferon.* rIFN (Schering, Kenilworth, NJ) was provided in lyophilized form and reconstituted to a final concentration of $5 \times 10^6$ IU/ml. A placebo that was identical to the rIFN in protein content, pH, tonicity, and appearance was also provided. The in-

terferon or placebo treatment was administered as a nasal spray by using a metered pump device that delivered 0.05 ml per spray. Each nostril was sprayed with 0.1 ml of medication twice each day.

*Virus inoculum pool.* The virus inoculum used in this study was a coronavirus 229E strain provided by Dr. D. A. J. Tyrrell (MRC Common Cold Unit, Salisbury, England). The inoculum pool consisted of nasal wash from a human volunteer with a coronavirus cold. This material was tested for the presence of pathogenic bacteria and then diluted in Earle's balanced salt solution to a titer of ~200 $TCID_{50}$/ml before inoculation into volunteers. Each volunteer was challenged by intranasal inoculation with 0.25 ml/nostril for a total inoculum of ~100 $TCID_{50}$.

*Symptom scoring.* Symptom scoring was done by a modification of a previously published method [8]. Each volunteer was asked to record symptom scores daily during the study. Symptoms such as fever, chills, headache, muscle ache, sneezing, sore throat, hoarseness, and cough and the nasal symptoms of rhinorrhea and nasal obstruction were judged as absent to severe by assigning a score of 0–3. Symptom criteria for a cold were a total symptom score of at least 5, in addition to either the presence of rhinorrhea on at least three of the six study days after coronavirus inoculation or the subjective impression of the volunteer that he had a cold.

*Isolation of virus.* Specimens for isolation of virus were collected daily by nasal wash for seven days after challenge. Each nostril was rinsed with 5 ml of PBS that was then mixed 3:1 (vol/vol) with four-times concentrated beef-heart infusion broth containing 4% fetal calf serum and antibiotics (vancomycin, gentamicin, and amphotericin B). Antibody to IFN sufficient to neutralize $10^4$ IU of rIFN was then added to each milliliter of sample. After incubation at room temperature for at least 30 min, 0.2 ml of sample was inoculated into each of two tube cultures of human embryonic lung fibroblast cells (MRC-5) maintained in Eagle's MEM containing 2% fetal calf serum. One tube from each specimen was placed in a stationary rack and the other in a roller drum; all tubes were incubated at 33 C. The tubes were examined ever other day for the development of CPE. Tubes with apparent CPE were passaged to fresh MRC-5 cells and again observed. Samples with CPE both in the original tube and after passage were considered positive for coronavirus. Isolates of virus were confirmed as coronavirus by ELISA. A nasal wash done before challenge was inoculated into cynomologous monkey kidney, human foreskin fibroblast, HEp-2, and mink lung cells to detect unsuspected viruses.

*ELISA for coronavirus antigen.* Antiserum to coronavirus 229E was produced in C3H mice and in guinea pigs. The mouse antibody was diluted 1:500 in 0.1 *M* sodium bicarbonate (pH 9.0) and adsorbed to the wells of polystyrene microtiter plates by incubation overnight at 4 C. Unreacted binding sites were blocked by incubating the plates for an additional hour with Eagle's MEM containing 10% fetal bovine serum. After rinsing with tap water, 50 μl of uninfected media or cell culture supernatant from a coronavirus isolate was placed into each antibody-coated well and diluted 1:3 with media. The samples were incubated for 1.5–2.0 hr at room temperature, and the plates were then washed three times with tap water. The second antibody (guinea pig antibody to 229E) was then added to each well and incubated for 1 hr at room temperature. After again washing the plate with tap water, we incubated the third antibody, horseradish peroxidase–conjugated rabbit antibody to guinea pig IgG (Cappell Laboratories, West Chester, Pa) in the plate for 1 hr at room temperature. The plate was again washed with tap water, and the substrate, 0.05% 1, 2-phenyldiamine in PBS (pH 7.4) with 0.03% $H_2O_2$, was added to each well and incubated for 20 min at room temperature. The absorbance was measured at a wavelength of 492 nm by using a spectrophotometer (Titertek Multiskan®; Flow Laboratories, McLean, Va). Specimens were considered positive if the absorbance was >2 SD above the mean absorbance of the negative control wells.

*Serology.* An assay for neutralizing antibody was done in 96-well microtiter plates. Serial twofold dilutions of serum were incubated with 17 $TCID_{50}$ of coronavirus 229E for five days at 33 C. Wells were examined for CPE, and the titer of neutralizing antibody was calculated. Volunteers were considered infected if there was at least a fourfold increase in titer of neutralizing antibody over the course of the study.

*Study design.* Assignment to treatment groups was done randomly and was double-blind for volunteers and investigators. All volunteers were treated with intranasal rIFN ($10^6$ IU twice a day) or placebo for one week before challenge with the study virus, on the day of challenge, and for one week after challenge. Thus, treated volunteers received a total of 30

$\times 10^6$ IU of rIFN over 15 days. Volunteers indicated that each dose was taken by marking a form at the time the medication was administered; these forms were checked by the study staff each time the volunteers were seen. Symptom scores were monitored beginning on the first day of rIFN or placebo treatment and continuing for three weeks. On the eighth day of interferon or placebo treatment, all volunteers were challenged with the study virus. Nasal washes for detecting shedding of virus were collected for seven days beginning on the day after challenge.

*Statistical analysis.* Proportions were compared by a one-sided Fisher's exact test. Symptom scores in the two groups of recipients were compared by a two-sided Mann-Whitney *U* test.

## Results

Twenty-seven volunteers received placebo and twenty-nine received rIFN. One of the placebo recipients reported a symptom score of 8 on the day of coronavirus inoculation and was excluded from further data analysis.

*Effect of rIFN on symptoms.* The use of rIFN significantly reduced the symptoms of coronavirus colds (table 1). Nineteen (73%) of the 26 placebo recipients met symptom-score criteria for a cold, compared with 12 (41%) of the 29 rIFN recipients ($P = .02$). The mean nasal symptom score for the six days after challenge was 9.2 in the placebo recipients and 5.4 in the rIFN recipients ($P = .03$). The total symptom scores in the two groups were 23.2 and 9.4, respectively ($P = .003$). The mean number of days with a total symptom score >4 was 1.6 days in the placebo recipients and 0.5 days in the rIFN recipients ($P = .02$). The peak day of symptoms was day 5 in the placebo recipients and day 4 in the rIFN recipients (figure 1). The mean total symptom scores were significantly different in the two groups on days 3, 4, 5, and 6 after challenge. No volunteers in either group developed symptoms after treatment was discontinued.

**Table 1.** The effect of prophylactic intranasal rIFN on the symptoms of coronavirus colds.

| Parameter | Treatment group: Interferon | Treatment group: Placebo | *P* |
|---|---|---|---|
| Met symptom criteria for a cold | 12/29 (41%) | 19/26 (73%) | .02* |
| Mean (± SD) nasal symptom score | 5.4 ± 5.3 | 9.2 ± 7.1 | .03† |
| Mean (± SD) total symptom score | 9.4 ± 8.6 | 23.2 ± 22.1 | .003† |
| Mean (± SD) no. of days with total symptom score >4 | 0.5 ± 0.9 | 1.6 ± 1.7 | .02† |

* Fisher's exact test (one-sided).
† Mann-Whitney *U* test (two-sided).

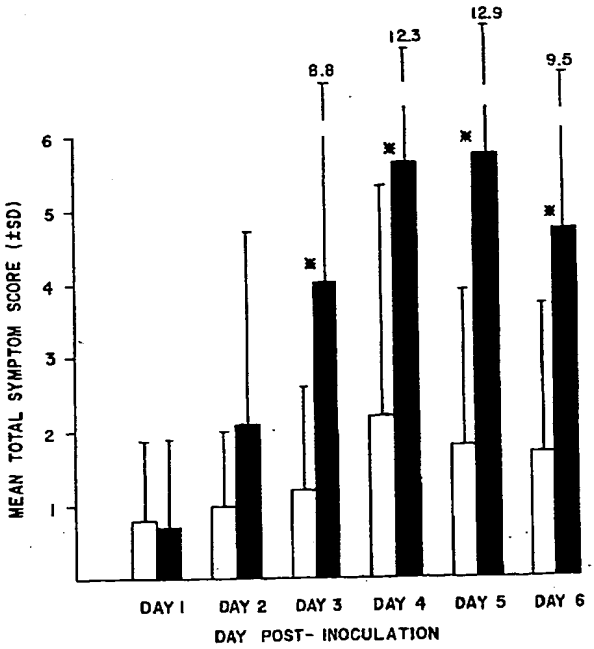


**Figure 1.** Effect of interferon (white columns) and placebo (black columns) on mean daily symptom scores during coronavirus colds. *, $P < .05$ for rIFN vs. placebo, by the Mann-Whitney *U* test.

*Effect of rIFN on infection.* The rate of viral infection was not altered by rIFN prophylaxis in this study (table 2). Twenty (77%) of the 26 placebo recipients and 23 (79%) of the 29 rIFN recipients had evidence of coronavirus infection. Eleven of these infections were diagnosed only by isolating virus, and 13 were detected only by serology. Nineteen volunteers had both an isolate of virus and a serological response to the virus. Nineteen (11%) of 174 cultures from placebo recipients and 19 (10%) of 198 cultures from rIFN recipients were positive for coronavirus. Ten (36%) of 28 volunteers in the treated group and eight (33%) of 24 volunteers in the placebo group had an initial serum titer of neutralizing antibody >1:32. These volunteers with high initial titers of antibody were significantly less likely

Table 2. The effect of prophylactic intranasal rIFN on coronavirus infection.

| Subjects with | Treatment group: Interferon ($n$ = 29) | Treatment group: Placebo ($n$ = 26) |
|---|---|---|
| Shedding of virus only | 7 | 4 |
| Seroconversion only | 6 | 7 |
| Both seroconversion and shedding of virus | 10 | 9 |
| Total infected | 23 (79%) | 20 (77%) |

NOTE. Data are no. of subjects.

to seroconvert than were those with lower initial titers. Four (22%) of 18 volunteers with an initial titer >1:32 had at least a fourfold increase in antibody titer, compared with 29 (85%) of 34 volunteers with an initial titer <1:32 ($P < .001$). Convalescent sera were not available for three volunteers. The presence of antibody in the acute serum specimens had no discernible effect on the rate of isolation of virus or on symptom scores.

*Side effects of rIFN.* Five placebo and 10 rIFN recepients reported bloody nasal mucus at least once during the 15 days of the study. Four placebo recipients and six rIFN recipients reported bloody nasal mucus in the first week of observation, before inoculation with coronavirus. These differences were not statistically significant. There was also no difference in nasal or total symptom scores in the two groups before challenge. Nasal speculum examinations done on days 1, 8, and 15 revealed no mucosal ulcerations in either of the treatment groups; however, bleeding sites were seen in eight (28%) of the rIFN recipients and in one (4%) of the placebo recipients ($P = .05$). Abnormalities in blood counts, blood chemistries, or urinalyses that could be attributed to rIFN treatment were not seen. No volunteers withdrew from the study due to side effects of the interferon or placebo treatment.

## Discussion

The results of this study indicate that prophylactic intranasal rIFN at a dose of $2 \times 10^6$ IU/day effectively reduces both the duration and severity of coronavirus colds. This beneficial effect on symptoms was not, however, associated with a decreased incidence of infection. In a previous study, Higgins et al. [7] gave $12 \times 10^6$ IU of intranasal interferon/day to volunteers beginning one day before coronavirus challenge. In that study, both infection and illness were reduced in the interferon-treated group. The ability of interferon to modify illness at doses that do not prevent infection has also been reported in studies of rhinovirus infection. Samo et al. [9] reported that 2.4 and $10 \times 10^6$ IU of interferon daily modified rhinovirus illness but did not affect the rate of viral infection. At higher daily doses of interferon, however, both illness and infection were significantly reduced. The mechanism by which symptoms are reduced by interferon without an associated reduction in infection is not known. No attempt was made in this study to quantify the amount of virus shed in the nasal secretions or to determine the effect of interferon on the host response to the virus. Either of these factors may be important in the production of symptoms during respiratory viral infection [10, 11].

The study of the human coronaviruses has been hampered by difficulties in isolating the virus from clinical specimens. In this study, 13 of the 26 placebo-treated volunteers had virus isolated from nasal wash specimens. Unlike many other viruses, the coronaviruses do not produce a distinctive CPE in cell culture and cannot be reliably distinguished from nonspecific cytotoxicity. To assure the specificity of the cell culture isolations, we routinely passaged cultures with apparent coronavirus CPE to fresh cells. Only those specimens that again produced CPE were considered positive for coronavirus when the data were analyzed. The positive cultures were confirmed as coronavirus by an ELISA. This evidence for the specificity of isolation of virus is further supported by the fact that 32 (74%) of the 43 volunteers with virus isolated also had a fourfold increase in titers of neutralizing antibody to the virus. Of the 11 patients who did not have a significant rise in titers of neutralizing antibody, nine had an initial titer ≥1:32, and the other two did not have convalescent sera available for analysis. An inverse relation between the frequency of seroconversion and the level of preexisting antibody to coronavirus 229E has been previously reported [12].

An interesting and important feature of coronavirus epidemiology is the ability of this virus to infect and produce symptoms associated with shedding of virus in spite of the presence of preexisting neutralizing antibody [12]. The presence of high titers of antibody had no effect on either illness or infection in this study. Volunteers with high initial titers of antibody were less likely to have a fourfold rise in titer,

an effect that has been previously reported. Reed [6] has recently reported that there are strain differences within the serogroup 229E and that infection may provide protective immunity to the homologous strain but not to heterologous strains.

The role that intranasal rIFN therapy can play in the prevention or treatment of coronavirus colds remains to be determined. Regimens that have been studied for preventing colds include prophylaxis during epidemic periods and contact prophylaxis in the home [3, 4, 13]. The lack of information about coronavirus epidemiology makes it difficult to predict if either or both of these regimens will be practical for preventing coronavirus infection. It is also not known whether rIFN will be effective for treating coronavirus colds. Further studies that address the issue of practical dosing regimens for preventing or treating the common cold are needed before rIFN can be useful in a clinical setting.

**References**

1. Dingle JH, Badger GF, Jordan WS Jr. Illness in the home: a study of 25,000 illnesses in a group of Cleveland families. Cleveland: Press of Western Reserve University, 1964:398
2. Gwaltney JM Jr, Hendley JO, Simon G, Jordan WS Jr. Rhinovirus infection in an industrial population. I. The occurrence of illness. N Engl J Med 1966;275:1261–8
3. Douglas RM, Moore BW, Miles HB, Davies LM, Graham NMH, Ryan P, Worswick DA, Albrecht JK. Prophylactic efficacy of intranasal alpha$_2$-interferon against rhinovirus infections in the family setting. N Engl J Med 1986;314: 65–70
4. Hayden FG, Albrecht JK, Kaiser DL, Gwaltney JM Jr. Prevention of natural colds by contact prophylaxis with intranasal alpha$_2$-interferon. N Engl J Med 1986;314:71–5
5. Gwaltney JM Jr. The common cold. In: Mandell GL, Douglas RG Jr, Bennett JE, eds. Principles and practice of infectious diseases. 2nd ed. New York: John Wiley and Sons, 1985:351–5
6. Reed SE. The behavior of recent isolates of human respiratory coronavirus in vitro and in volunteers: evidence of heterogeneity among 229E-related strains. J Med Virol 1984;13:179–92
7. Higgins PG, Phillpotts RJ, Scott GM, Wallace J, Bernhardt LL, Tyrrell DAJ. Intranasal interferon as protection against experimental respiratory coronavirus infection in volunteers. Antimicrob Agents Chemother 1983;24:713–5
8. Jackson GG, Dowling HF, Spiesman IG, Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. Arch Intern Med 1958;101:267–78
9. Samo TC, Greenberg SB, Palmer JM, Couch RB, Harmon MW, Johnson PE. Intranasally applied recombinant leukocyte A interferon in normal volunteers. II. Determination of minimal effective and tolerable dose. J Infect Dis 1984;150:181–8
10. Douglas RG Jr, Cate TR, Gerone PJ, Couch RB. Quantitative rhinovirus shedding patterns in volunteers. Am Rev Respir Dis 1966;94:159–67
11. Gwaltney JM Jr, Hendley JO, Mygind N. Symposium on rhinovirus pathogenesis: summary. Acta Otolaryngol [Suppl] (Stockh) 1984;413:43–5
12. Hamre D, Beem M. Virological studies of acute respiratory disease in young adults. V Coronavirus 229E infections during six years of surveillance. Am J Epidemiol 1972;96: 94–106
13. Monto A, Shope T, Schwartz S, Albrecht J. Clinical trials of IFN-alpha$_2$ (SCH 30500) for prophylaxis of naturally occurring respiratory infection [abstract WS-19-9]. In: 14th International Congress of Chemotherapy: abstracts. Kyoto, Japan: International Society of Chemotherapy, 1985

This Page Blank (uspto)